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Morphometry and geographical variation of *Bothriurus bonariensis* (Scorpiones: Bothriuridae)

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Abstract. Diversification of morphological characteristics among geographically separated populations is particularly important in understanding evolutionary processes and is considered the early stage of allopatric speciation. In the present study, we investigated geographic variation in scorpion populations of *Bothriurus bonariensis* (Koch 1842). Our principal goal was to compare different populations of this species with regard to its distribution, analyzing somatic and genitalic characters. In Argentina, specimens of *B. bonariensis* from Entre Ríos and Corrientes Provinces are dark brown, while specimens from La Pampa have reddish coloration. Scorpions of this species from Brazil and south of Uruguay are totally black. Also, we observed variability in body size, some morphological characteristics of adult specimens (e.g., prosoma length, hand height, hand width, and telson height), and genitalic traits (e.g., hemispermatophore lamina length, basal and distal lamina width, dorsal fold length). Results indicate the presence of evident geographic variation: populations from Argentina show similar morphological patterns that differ from populations from Uruguay. We discuss these data in the context of the current phylogeographical and evolutionary knowledge of this species.

Keywords: Coloration, genitalia, scorpion

Diversification of morphological characteristics among geographically separated populations is particularly important and is considered the starting point of allopatric speciation. For this reason, data on this diversification process are useful for understanding evolutionary processes (Coyne and Orr 2004). Differences among habitats can generate a divergent selection of groups in nature, resulting in reproductive isolation of the populations (Mayr 1942, 1963; Schluter 2000). Although geographical variation of genitalic traits can also be very important at morphological and behavioral levels, the analysis of this important aspect has received less attention (Eberhard 1985; Reinhardt 2010). The environment can influence species directly or indirectly with regard to genitalic variation; therefore, it is expected that these features can vary in shape or size in different populations or geographic locations. Although it is difficult to identify geographic factors that cause these variations and to separate these factors from genotypic effects, it is known that local and clinal variations in genitalic characters do exist (Lachaise et al. 1981; Hribar 1994; Tatsuta & Akimoto 1998; Kelly et al. 2000; Jennions & Kelly 2002; Tatsuta et al. 2001). Studies performed in different groups of insects and arachnids have shown that populations of a single species can begin differentiating both morphologically and genetically as a result of allopatric distribution, reaching reproductive isolation afterwards (Yamashita & Polis 1995; Postiglioni & Costa 2006; Holwell 2008).

The present study approaches this subject in populations of the type species of the scorpion family Bothriuridae: *Bothriurus bonariensis* (Koch 1842). This species has a broad distribution in South America. It inhabits central Argentina, Uruguay, and southeastern Brazil (Mattoni & Acosta 2005; Ojanguren Affilastro 2005) (Fig. 1). In Argentina, this scorpion can be found in the Provinces of Buenos Aires, La Pampa, Córdoba, San Luis, Entre Ríos, Corrientes and possibly Santa Fe. This distribution corresponds to the Pampean and Espinal phytogeographical provinces (Cabrera & Willink 1980; Ojanguren Affilastro 2005).

Within its range, some variations occur in the coloration patterns of specimens from different zones. In Argentina, specimens from the Provinces of Entre Ríos and Corrientes are dark brown, while specimens from La Pampa province have reddish coloration, with large areas lacking dark or blackish pigment. In contrast, specimens from Brazil and Uruguay are totally black (Mattoni 2003; Ojanguren Affilastro 2005). This notable variability in pigmentation was mentioned by San Martín (1962) for different populations in Uruguay. Similarly, great variability in body size of adult specimens has been observed. Individuals from the Espinal area of Córdoba are remarkably smaller than those from Uruguay and Brazil (Mattoni 2003). As in other animal groups (Alatalo et al. 1988; Moller 1991; Andersson 1994; Pomiankowski & Moller 1995), in this species there is great phenotypic variation in characters under sexual selection. For example, some structures of the spermatophore such as the lamina crest and the lateral fold show high coefficients of variation (Peretti et al. 2001). In addition, spermatophores and hemispermatophores of male *B. bonariensis* from Córdoba (at the margin of the range of the species) show higher fluctuating asymmetry than specimens from the southern part of Buenos Aires Province (Peretti et al. 2001; Peretti unpublished data). Nevertheless, these differences have not been compared with populations from other areas.

The main objective of this work is to compare different populations of *B. bonariensis* within its range (from Córdoba, Entre Ríos, and Buenos Aires Provinces in Argentina and north and south of Uruguay) by analyzing somatic and genitalic characters. We expected to find morphometric variation among populations that was related to the general geographical distribution of the species.

METHODS

Study Material.—We used specimens from the following collections: Museo Argentino de Ciencias Naturales, Buenos

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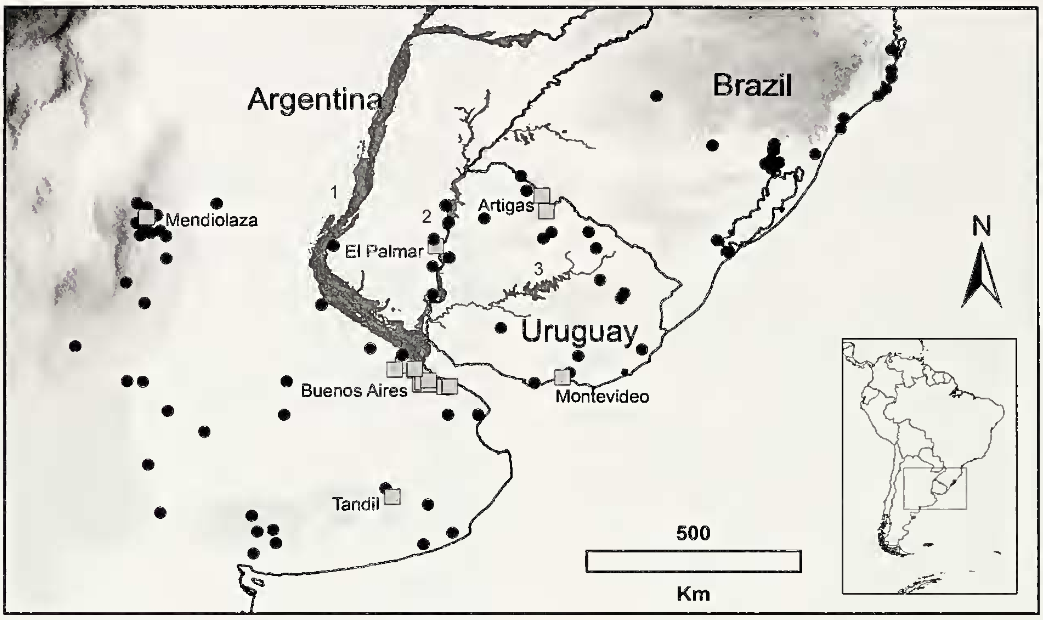

Figure 1.—Geographic distribution of *Bothriurus bonariensis*. Black circles indicate the total distribution of the species known to date; gray squares show the populations used in this work. Major water bodies are displayed in gray (1: Paraná River, 2: Uruguay River, 3: Negro River).

Aires (MACN); Laboratorio de Biología Reproductiva y Evolución, Córdoba (LBRE); and A. V. Peretti's personal collection (AVP). We analyzed speeimens of *B. bonariensis* that were selected from different distant populations (Fig. 1). We only selected populations from which materials were abundant (allowing statistical comparisons) and well preserved (poor preservation techniques alter the pigmentation pattern and the hemispermatophore form). These populations were examined: Mendiolaza (Córdoba Province, Argentina – 31°16′0.01″S, 64°18′0.04″W, 543 m.a.s.l.): 20 males, 11 females, 20 pairs of hemispermatophores; Tandil (Buenos Aires, Argentina – 37°19′4.12″S, 59°09′1.41″W, 190 m.a.s.l.): 7 males, 3 females, 6 pairs of hemispermatophores; La Plata and surroundings of Buenos Aires (Buenos Aires Province, Argentina – 34°36′30.3″S, 58°22′23.38″W, 26 m.a.s.l.): 10 males, 5 females, 8 pairs of hemispermatophores; El Palmar National Park (Entre Ríos Province, Argentina – 31°51′11″S, 58°19′21″W, 21 m.a.s.l.): 8 males, 9 females, 8 pairs of hemispermatophores; Artigas and surroundings (Artigas Province, Uruguay – 30°29′17.12″S, 57°6′4.75″W, 85 m.a.s.l.): 9 males, 3 females, 7 pairs of hemispermatophores; Piedras de Afilar (Canelones Province, Uruguay – 34°44′S, 55°35′W, 57 m.a.s.l.): 5 males, 4 females, 5 pairs of hemispermatophores (Fig. 1) (complete list of materials in Appendix I).

Characterization of the population.—*Preparation of the sample and criteria for morphological analysis:* To compare different populations of *B. bonariensis*, we followed the biological species concept (Mayr 1942). All specimens studied were preserved in 80% ethanol. Hemispermatophores were extracted, cleaned and preserved following Sissom et al. (1990). Color characteristics and patterns of pigmentation between populations were compared based on San Martín (1962) and Ojanguren Affilastro (2005). Measurements of quantitative characters of external morphology were taken on the right side in both males and females and on the left hemispermatophore in males (Peretti et al. 2001). The measurements were taken with a Nikon SMZ1500 stereomicroscope with ocular micrometer, equipped with a Nikon Sight DS-Fi1 digital camera. Digital pictures were analyzed with the Image Tool 3.0 measuring software (© UTHSCSA 1996–2002). We examined 28 morphometric characters (20 in males, 8 in females) (Table 1, Fig. 2).

Morphometrics: We performed all the statistical analyses with the PC program NCSS 2007 (© Hintze 2007). Results of measurements of each character were analyzed with analysis of variance tests (ANOVA or Kruskal-Wallis one-way ANOVA, depending of the normality of the data) and a posteriori tests (Tukey-Kramer or Dunn). Although it is unknown whether post-reproductive molting after reaching the adult stage occurs in scorpions (Polis & Sissom 1990), the adults of

Table 1.—Body and hemispermatophore characters measured in the *Bothriurus bonariensis* populations. The numbers of each character correspond to those shown in Figure 2.

Body Characters	Length	Width	Height	Calculated proportions
Prosoma	1			1/2
Hand	2	3	4	1/4
Movable finger	5			2/4
Telson	6		7	2/5
Pecten	8			6/7
Hemispermatophores				
Lamina	9	10 (distal) 11 (median) 12 (basal)		1/9+13
Trunk	13			9/11
Capsular lobe	14	15		9/13
Dorsal fold	16	17		14/9+13
Dorsal fold-Lateral edge space	18			18/9
Lateral edge	19			20/9
Crest	20			

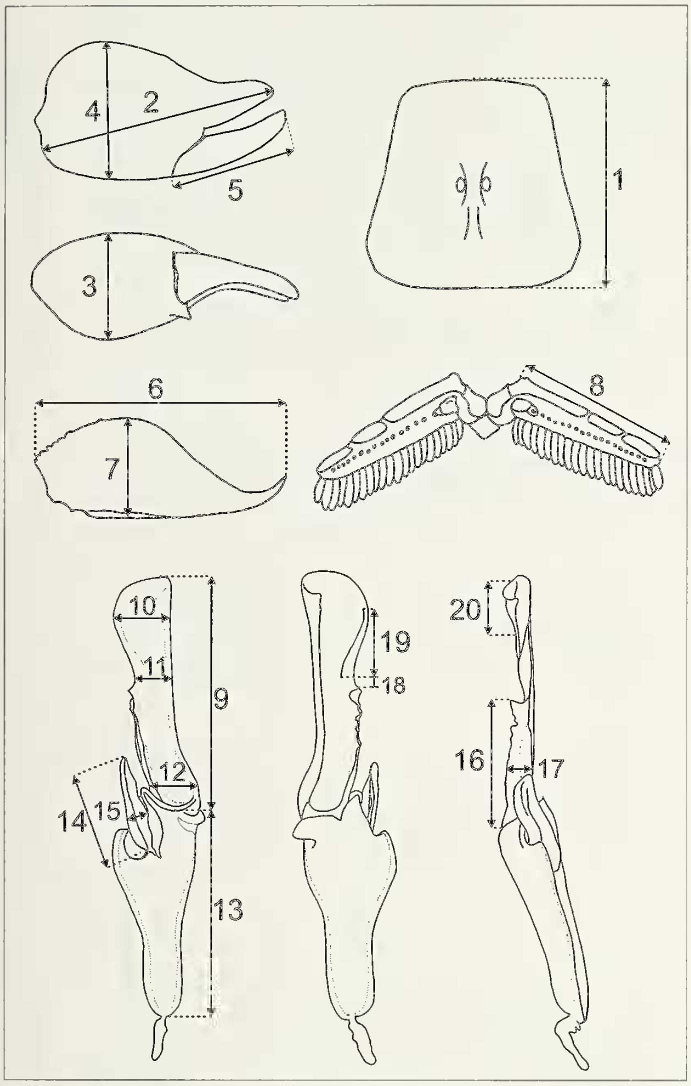

Figure 2.—Body and genitalic characters measured in the samples. The numbers refer to character number cited in Table 1.

B. bonariensis show a wide range of sizes (Peretti et al. 2001), as observed in other scorpions and spiders (Savory 1977; Polis & Sissom 1990; Benton 1992). For this reason, besides comparing the mean values of absolute measurements for each character, we calculated indices that show proportions of some of the body traits compared to others. These proportion values are shown in Table 1.

Multivariate analyses: To compare populations, including all the selected variables, we performed a multivariate discriminant analysis (Fisher 1936). Discriminant analysis results in a set of prediction equations (linear functions) based on independent variables that are used to classify individuals into groups. As an estimating method, the discriminant linear function was used without setting a priori probabilities and without selecting variables. Canonical Correlation Coefficient and Wilks' Lambda were calculated, which allowed us to choose the most appropriate model among all models that can be constructed from the variables previously selected. Both ratios measure the differences between the groups due to discriminant functions. This technique allowed us to analyze whether there was a separation among populations, considering the variables studied, and to determine the influence of each variable on the population discrimination level (Brown & Wicker 2000; Vignoli et al. 2005).

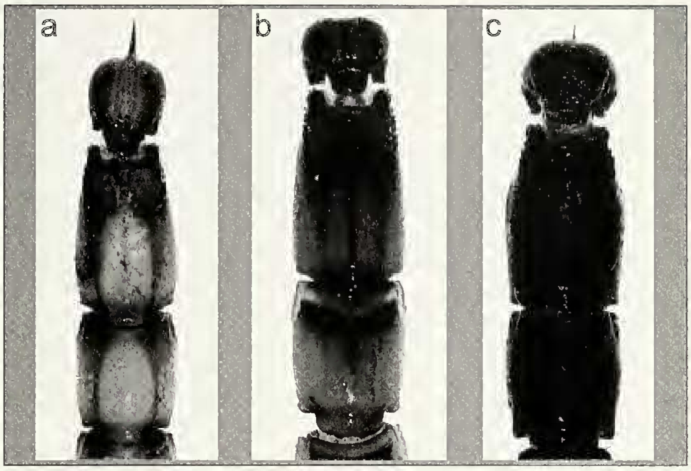

Figure 3.—Pigmentation patterns found on the ventral side of the metasoma in different populations of *Bothriurus bonariensis.* a) two darker lateral lines in all segments; b) two darker lines, one central line in the fifth segment and/or central patches in all segments; c) dark uniform coloration in all segments.

RESULTS

External morphological analysis.—We observed remarkable variation in the color of the specimens from different populations. Coloration of individuals in the Mendiolaza population was brownish-gray, the same as in individuals from the Buenos Aires and Tandil populations. Specimens from the El Palmar population showed dark and reddish coloration, which was darker in the specimens from Artigas population. Piedras de Afilar specimens were totally black. There were three different pigmentation patterns in the metasoma ventral area: A) two dark lateral lines in all segments of the metasoma; B) two dark lines, one central line in the fifth segment and/or central patches in all the segments; or C) dark uniform coloration in all the segments (Fig. 3).

Scorpions from the Mendiolaza and Tandil populations manifested Pattern A in all cases. Individuals from Piedras de Afilar showed only Pattern C. Specimens from Buenos Aires and El Palmar displayed different percentages of Patterns A and B (Buenos Aires: 60% A, 40% B; El Palmar: 50% A, 50% B), whereas in the population from Artigas, all three patterns occurred (40% A, 25% B, 35% C).

Morphometric analysis.—Varying body measurements reflected separation of the populations studied in three groups: specimens from Mendiolaza were smaller, specimens from Piedras de Afilar were larger, and specimens from other populations showed intermediate sizes (Tables 2, 3).

Body index also indicated significant division among populations in almost all the variables (Table 4). The relative height of the hand in relation to hand length (hand height/hand length) in males and females was greater in individuals from Piedras de Afilar (Figs. 4a, b). Similarly, length and height of the hands were greater in relation to prosoma length in Piedras de Afilar compared to other populations. These variables

Table 2.—Mean values (mm) and standard deviations of the male's body and hemispermatophore traits measured in six populations of *Bothriurus bonariensis*. Separation according to ANOVA test (F statistic) and Kruskal-Wallis test (H statistic). A, B, and C indicate the grouping and separation of populations by Tukey-Kramer and Dunn tests.

N°	Body characters	Mendiolaza	Tandil	La Plata and Buenos Aires suburbs	El Palmar National Park	Artigas and surrounds	Piedras de Afilar	*df*	Statistics	*P*
1	Prosoma length	5.26 ± 0.10	5.59 ± 0.17	5.52 ± 0.14	5.75 ± 0.16	5.53 ± 0.15	6.08 ± 0.20	5, 58	F = 3.34	0.01
		A	AB	AB	AB	AB	B			
2	Hand length	7.22 ± 0.12	7.71 ± 0.20	7.69 ± 0.17	7.55 ± 0.19	7.52 ± 0.18	7.78 ± 0.24	5, 58	H = 9.13	0.1
3	Hand width	2.38 ± 0.06	2.87 ± 0.10	2.85 ± 0.08	2.66 ± 0.10	2.69 ± 0.09	3.17 ± 0.12	5, 58	H = 28.80	<0.001
		A	B	B	AB	AB	B			
4	Hand height	3.50 ± 0.08	4.20 ± 0.14	4.08 ± 0.12	4.09 ± 0.13	4.09 ± 0.12	4.60 ± 0.17	5, 58	H = 29.85	<0.001
		A	B	B	B	B	B			
5	Movable finger length	3.76 ± 0.06	4.16 ± 0.10	4.15 ± 0.08	4.15 ± 0.10	4.15 ± 0.09	4.02 ± 0.12	5, 58	H = 20.53	<0.001
		A	AB	B	B	B	AB			
6	Telson length	6.04 ± 0.10	6.50 ± 0.17	6.53 ± 0.15	6.43 ± 0.16	6.23 ± 0.15	7.24 ± 0.21	5, 58	H = 20.35	0.001
		A	AB	AB	AB	AB	B			
7	Telson height	1.84 ± 0.04	2.22 ± 0.07	2.20 ± 0.06	2.12 ± 0.07	2.05 ± 0.07	2.83 ± 0.09	5, 58	H = 33.16	<0.001
		A	B	B	AB	AB	B			
8	Pecten length	4.66 ± 0.15	4.74 ± 0.26	4.71 ± 0.24	5.01 ± 0.24	4.96 ± 0.23	4.94 ± 0.31	5, 56	H = 12.33	0.03
	Genitalic characters									
9	Lamina length	5.11 ± 0.06	5.78 ± 0.10	5.53 ± 0.08	5.55 ± 0.10	6.11 ± 0.09	6.16 ± 0.11	5, 50	F = 26.20	<0.001
		A	BC	C	C	B	B			
10	Lamina width (distal)	1.51 ± 0.03	1.65 ± 0.05	1.57 ± 0.04	1.61 ± 0.05	1.66 ± 0.09	1.69 ± 0.11	5, 50	F = 3.58	0.008
		A	AB	AB	AB	AB	B			
11	Lamina width (median)	0.99 ± 0.02	1.04 ± 0.04	1.01 ± 0.03	0.99 ± 0.04	1.08 ± 0.04	1.10 ± 0.04	5, 51	F = 1.62	0.17
12	Lamina width (basal)	1.20 ± 0.02	1.29 ± 0.03	1.23 ± 0.03	1.25 ± 0.03	1.36 ± 0.03	1.37 ± 0.04	5, 51	F = 5.89	<0.001
		A	AB	AC	AB	B	BC			
13	Trunk length	4.31 ± 0.07	4.29 ± 0.18	4.53 ± 0.13	4.54 ± 0.13	4.42 ± 0.12	5.09 ± 0.14	5, 46	H = 13.97	0.01
		A	AB	AB	AB	AB	B			
14	Capsular lobe length	2.46 ± 0.05	2.34 ± 0.08	2.42 ± 0.07	2.36 ± 0.08	3.00 ± 0.08	2.50 ± 0.09	5, 51	H = 13.13	0.02
		A	B	B	B	B	B			
15	Capsular lobe width	0.49 ± 0.01	0.48 ± 0.02	0.49 ± 0.02	0.47 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	5, 51	H = 15.95	0.006
		AB	AB	AB	A	B	AB			
16	Dorsal fold length	2.49 ± 0.04	2.99 ± 0.08	2.78 ± 0.07	2.78 ± 0.08	3.50 ± 0.08	3.19 ± 0.09	5, 51	H = 40.06	<0.001
		A	B	AB	AB	B	B			
17	Dorsal fold width	0.60 ± 0.01	0.61 ± 0.02	0.64 ± 0.02	0.62 ± 0.02	0.66 ± 0.02	0.69 ± 0.03	5, 51	H = 11.24	0.04
		A	AB	AB	AB	AB	B			
18	Distance of dorsal fold–lateral edge	0.47 ± 0.04	0.29 ± 0.07	0.38 ± 0.06	0.22 ± 0.07	0.16 ± 0.06	0.42 ± 0.07	5, 50	F = 4.87	0.001
		A	AB	AB	B	B	AB			
19	Lateral edge length	1.23 ± 0.05	1.60 ± 0.09	1.37 ± 0.07	1.60 ± 0.09	1.60 ± 0.08	1.40 ± 0.09	5, 50	H = 20.74	<0.001
		A	B	AB	B	B	AB			
20	Crest length	0.95 ± 0.05	1.08 ± 0.08	1.03 ± 0.07	1.06 ± 0.08	1.07 ± 0.08	1.06 ± 0.09	5, 49	H = 10.78	0.06

Table 3.—Comparison of females of different populations of *Bothriurus bonariensis* in terms of average values (mm) and standard deviations of the variables used. Separation according to ANOVA test (F statistic) and Kruskal-Wallis (H statistic). The grouping and separation of populations by Tukey-Kramer and Dunn tests is indicated by A, B, and C.

N°	Body characters	Mendiolaza	Tandil	La Plata and Buenos Aires suburbs	El Palmar National Park	Artigas and surrounds	Piedras de Afilar	*df*	Statistical	*P*
1	Prosoma length	5.2 ±0.18	5.50 ±0.35	5.81 ±0.27	5.19 ±0.20	6.26 ±0.35	5.51 ±0.30	5, 34	F = 2.12	0.09
2	Hand length	6.87 ± 0.20 B	7.00 ± 0.38 AB	7.47 ± 0.33 AB	6.76 ± 0.21 B	8.26 ± 0.38 A	6.60 ± 0.33 B	5, 33	F = 3.27	0.01
3	Hand width	1.98 ± 0.07 B	2.21 ± 0.14 AB	2.37 ± 0.11 AB	2.12 ± 0.08 B	2.73 ± 0.14 A	2.18 ± 0.12 AB	5, 34	F = 5.44	0.001
4	Hand height	2.72 ± 0.10 B	2.94 ± 0.20 AB	3.18 ± 0.16 AB	2.99 ± 0.12 B	3.77 ± 0.20 A	3.00 ± 0.17 AB	5, 34	F = 4.54	0.003
5	Movable finger length	3.69 ± 0.11 B	3.99 ± 0.21 AB	4.03 ± 0.16 AB	3.68 ± 0.12 B	4.68 ± 0.21 A	3.48 ± 0.18 B	5, 34	F = 4.96	0.002
6	Telson length	5.32 ± 0.17 B	5.43 ± 0.32 AB	5.43 ± 0.19 AB	6.04 ± 0.25 B	6.76 ± 0.32 A	5.61 ± 0.28 AB	5, 34	F = 3.93	0.007
7	Telson height	1.67 ± 0.07 A	1.97 ± 0.14 ABC	2.11 ± 0.11 BC	1.88 ± 0.08 AC	2.44 ± 0.14 B	1.98 ± 0.12 ABC	5, 34	F = 5.45	0.001
8	Pecten length	3.65 ± 0.14	3.89 ± 0.28	4.03 ± 0.24	3.87 ± 0.17	4.64 ± 0.28	3.90 ± 0.24	5, 32	F = 2.03	0.11

Table 4.—Body indices calculated for male and female populations of *Bothriurus bonariensis*. Separation according to ANOVA test (F statistic) and Kruskal-Wallis (H statistic).

Indices of proportions	*df*	Statistical	*P*
Males			
Prosoma length/Hand length	5, 58	H = 11.49	0.04
Hand length / Hand height	5, 58	F = 22.13	<0.001
Hand length /Movable finger length	5, 58	F = 4.65	0.001
Prosoma length / Hand height	5, 58	H = 30.65	<0.001
Telson length/ Telson height	5, 58	F = 12.41	<0.001
Prosoma length /Hemispermatophore length	5, 45	H = 12.01	0.03
Lamina length/ Lamina width (median)	5, 50	F = 4.64	0.002
Lamina length /Trunk length	5, 45	H = 19.48	0.002
Capsular lobe length/ Hemispermatophore length	5, 45	H = 11.77	0.04
Dorsal fold length/ Lamina length	5, 50	F = 13.41	<0.001
Crest length/ Lamina length	5, 49	H = 2.02	0.8
Females			
Prosoma length / Hand length	5, 33	H = 11.55	0.04
Hand length / Hand height	5, 33	H = 23.93	<0.001
Hand length / Movable finger length	5, 33	F = 3.54	0.01
Prosoma length / Hand height	5, 34	F = 2.55	0.05
Telson length / Telson height	5, 34	H = 11.87	0.04

signify that the hands were larger and broader, giving a more robust aspect to the specimens from the Piedras de Afilar population. In the Mendiolaza population, these structures were slimmer. The telson was also narrower related to its length (telson width/length) in Mendiolaza and more spherical in specimens from Piedras de Afilar (Figs. 4c, d). The other populations showed overlap among them, but were different in comparison with the Mendiolaza and Piedras de Afilar populations.

In male genitalia, indices in relation to prosoma length showed that the hemispermatophores in the Piedras de Afilar population were longer compared to the other populations (Fig. 5a). The lamina was shorter and broader in the Mendiolaza population and longer and slimmer in the individuals from the other populations (Fig. 5c). The capsular lobe was also longer compared to the total length of the hemispermatophore in the Piedras de Afilar population and shorter in the Mendiolaza population. The same pattern occurred with the dorsal fold of the lamina in relation to the lamina length in the Artigas population (Figs. 5b, d).

Multivariate analysis.—The discriminant multivariate analysis showed that, considering all the variables, there was a clear discrimination among the populations. In males, we found a significant separation between the Mendiolaza and Uruguay populations. These populations differed from the rest and between themselves as well. The Buenos Aires, Tandil, and El Palmar populations comprised a single group. The major discrimination occurred in the space of 1 and 2 scores for which the Wilk's Lambda values were 0.00085 and 0.016, respectively (Canonical discriminant analysis; *F*: 3.3 and 2.1, $P < 0.001$; Fig. 6a). For the rest of the scores, there was no separation.

As shown in Table 5, the variables that most influenced separation among males were two genitalic traits, the dorsal fold length and the lamina length of the hemispermatophore,

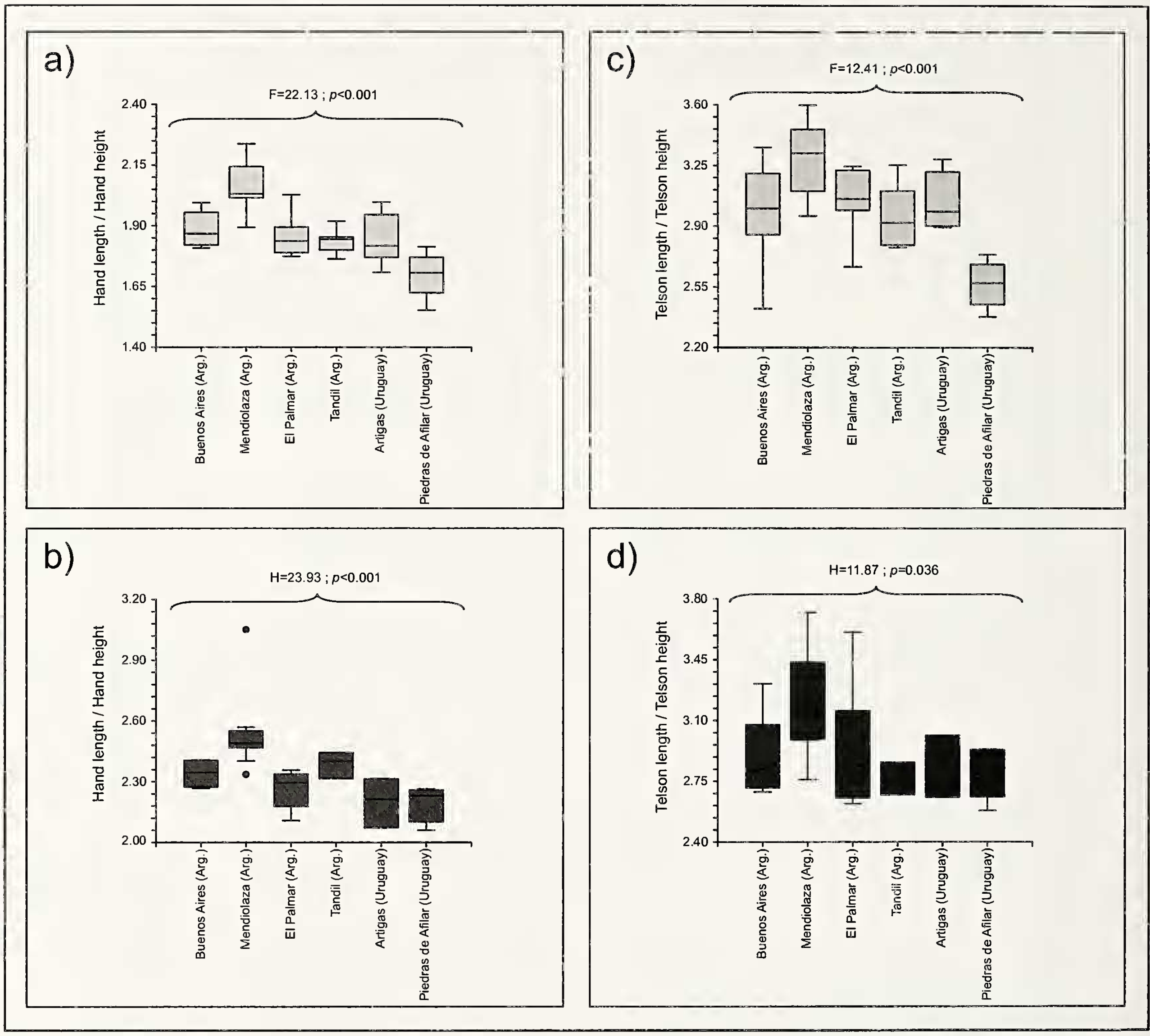

Figure 4.—Separation by means of box plots in *Bothriurus bonariensis* populations: a) hand length vs. hand height in males (ANOVA, $F_{5,58}$ = 22.13, $P < 0.001$); b) hand length vs. hand height in females (Kruskal-Wallis One-Way ANOVA, $H_{5,33}$ = 23.93, $P < 0.001$); c) telson length vs. telson height in males (ANOVA, $F_{5,58}$ = 12.41, $P < 0.001$); d) telson length vs. telson height in females (Kruskal-Wallis One-Way ANOVA, $H_{5,34}$ = 11.87, P = 0.036).

followed by body variables such as telson height and hand height and width.

In females, the differences were not so striking. With regard to males, the Mendiolaza population comprised one group, the Piedras de Afilar population another group, and the remaining populations a third group.

Scores that better explained the variation were 1 and 2 with Wilk's Lambda values of 0.018 and 0.08, respectively (Canonical discriminant analysis; F: 3.2 and 2.6, $P < 0.001$; Fig. 6b). Female variables that most influenced population differentiation were telson height and characters such as hand width and hand height, and length of the movable finger (Table 5).

DISCUSSION

Results indicate that intraspecific geographic variation occurs in *B. bonariensis*, both in morphological and genitalic characters. Populations from Argentina share morphological features that are notably different from features of the Uruguayan populations. In Argentina, the specimens from the Mendiolaza population are smaller and present slimmer and more delicate structures. Individuals from this population also have shorter hemispermatophores as well as shorter capsular lobes and dorsal folds, but broader lamina. The other populations in Argentina (Tandil, Buenos Aires, and El Palmar) do not differ among themselves, but comprise a different group (larger body size and slightly darker coloration).

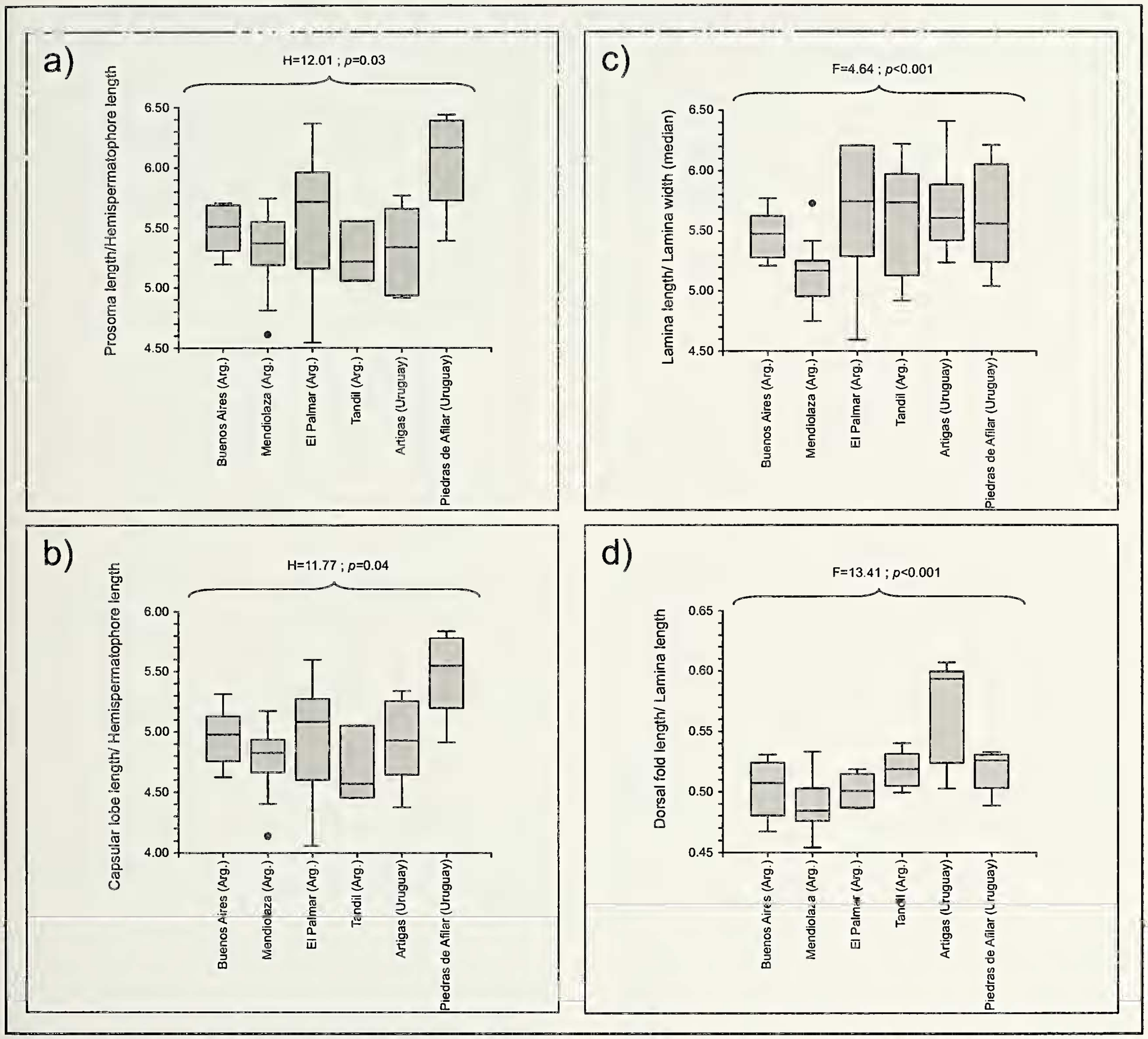

Figure 5.—Differences in male genitalia in the six populations of *Bothriurus bonariensis*: a) prosoma length vs. hemispermatophore length (Kruskal-Wallis One-Way ANOVA, $H_{5,45}$ = 12.01, P = 0.03); b) capsular lobe length vs. hemispermatophore length (Kruskal-Wallis One-Way ANOVA, $H_{5,45}$ = 11.77, P = 0.04); c) lamina length vs. lamina width (ANOVA, $F_{5,50}$ = 4.64, $P < 0.001$); d) dorsal fold length vs. lamina length (ANOVA, $F_{5,50}$ = 13.41, $P < 0.001$).

Scorpions from the Uruguay populations are more robust. The hemispermatophores in the Piedras de Afilar population are larger and possess slimmer and longer laminas. Specimens from northern Uruguay (Artigas) differ in size and, in agreement with San Martin (1962), their color is different from that in the southern Uruguay population (i.e., Piedras de Afilar). Indeed, The Uruguayan populations are larger with more spherical structures and completely black coloration (specimens from Artigas are reddish). Their hemispermatophores have shorter lamina and longer capsular lobes.

According to the discriminant analysis, genitalic variables in males allow for better differentiation among populations. This is very important from taxonomic and evolutionary points of view, because when high sexual selection pressures occur, genitalic traits tend to show rapid divergence compared to other morphological traits (Eberhard 1985, 2010; Hosken & Stockley 2003).

Regarding pigmentation variation, in the Mendiolaza and Tandil populations all specimens show the same pattern of two darker lateral lines in the ventral zone of the metasoma. In addition, the Buenos Aires and El Palmar populations display another pattern of dark lateral lines and many central lines. In contrast, the Piedras de Afilar specimens manifest a uniform dark coloration. Specimens from the Artigas population show

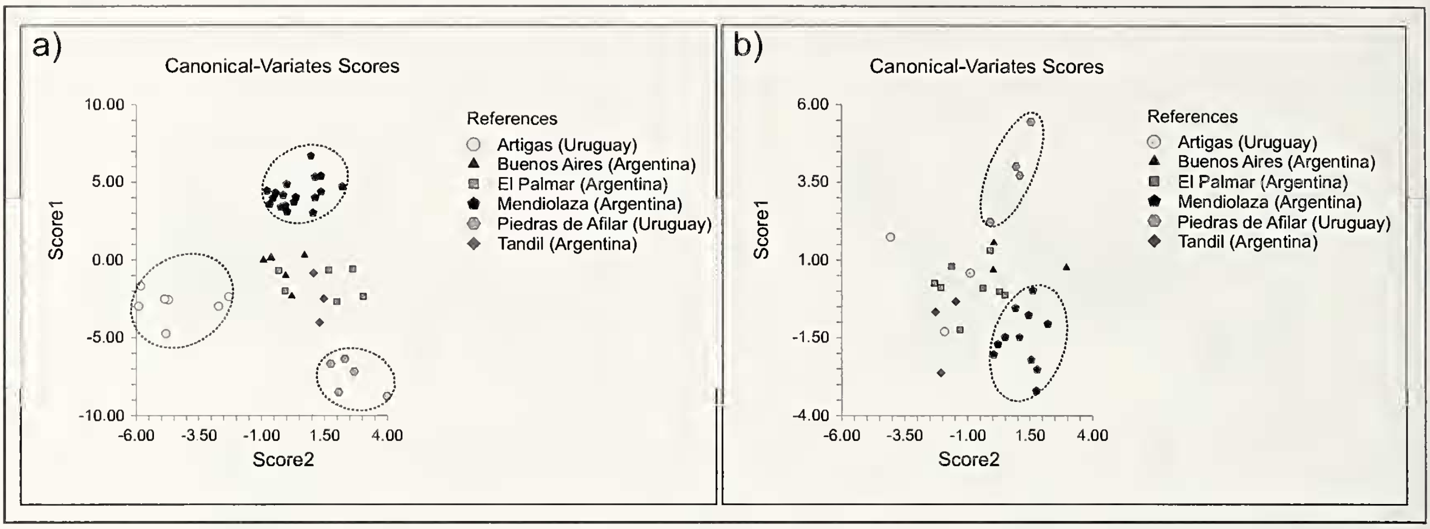

Figure 6.—Discrimination of populations of *Bothriurus bonariensis* in the space of scores 1 and 2 as Canonical Discriminant Analysis: a) males: Score 1: F: 3.3, $P < 0.001$, Wilk's lambda: 0.00085; Score 2: $F = 2.1$, $P < 0.001$, Wilk's lambda: 0.016; b) females: Score 1: F: 3.2, $P < 0.001$, Wilk's lambda: 0.018; Score 2: $F = 2.6$, $P < 0.001$, Wilk's lambda: 0.08.

all three patterns of pigmentation. Thus, not only the general coloration but also pigmentation patterns turn gradually more pigmented from Mendiolaza to Uruguay. This variation could be related to environmental characteristics that change throughout the species' range. The climate gets wetter and precipitation levels increase from west to east (data from Servicio Metereologico Nacional, online at www.smn.gov.ar, and Direccion Nacional de Metereologia de Uruguay, online at www.metereologia.com.uy). It is known that scorpion species belonging to wet zones show a greater amount of pigment than species inhabiting drier zones (Lourenço & Cloudsley-Thompson 1996; Mattoni 2002), and in this case, the patterns found in the present study could be caused for the same reason.

Table 5.—Influence of variables considered in the Canonical Discriminant Analysis for group separation in males and females of the studied populations of *Bothriurus bonariensis*.

Influence of variables in males		
Variable	F	P
Males		
Dorsal fold length	29.39	<0.001
Lamina length	28.71	<0.001
Telson height	22.81	<0.001
Hand height	11.25	<0.001
Hand width	9.22	<0.001
Capsular lobe length	8.95	<0.001
Movable finger length	6.52	<0.001
Telson length	6.26	<0.001
Lamina width (basal)	5.65	<0.001
Lateral edge length	5.46	<0.001
Trunk length	5.15	0.001
Distance of dorsal fold–lateral edge	4.05	0.004
Prosoma length	3.80	0.007
Lamina width (distal)	3.24	0.015
Capsular lobe width	2.90	0.025
Dorsal fold width	2.49	0.047
Females		
Telson height	5.45	0.001
Hand width	5.00	0.002
Movable finger length	4.45	0.005
Hand height	4.26	0.006
Telson length	3.49	0.015
Hand length	3.09	0.026

In consideration of all these patterns, variability in morphology of specimens from different populations related to the total distribution of the species could result in a gradient of size, coloration, morphological features (prosoma length, hand height and width, movable finger length and telson height), and genitalic traits (lamina length, basal and distal lamina width, dorsal fold length). This gradient may indicate that scorpions become larger and acquire more robust structures and darker coloration in populations located more eastward.

However, it is not possible to confirm the existence of an environmental cline or morphological gradation along the distribution of the species until new and abundant materials from intermediate populations become available. The samples from intermediate locations between the populations studied in Argentina are scarce, and most of them are extremely old and poorly preserved (preservation alters the pigmentation pattern and the hemispermatophore form). In fact, most of the intermediate localities that the species inhabit are now strongly anthropized, this region being the main agriculture area in Argentina (the "pampas," with more than 60% of the land planted with permanent crops; see www.fao.org for map). Almost all of the natural habitats in the humid "pampas" and "espinal" region have disappeared (Dinerstein et al. 1995), and finding abundant populations of fossorial scorpions, such as *B. bonariensis*, is difficult.

It is known that scorpions have limitations in moving great distances and adapting to particular types of environments (soils, climatic conditions, and vegetation: Polis 1990; Prendini 2001). Therefore, historical biogeographic processes could have led to population differentiation in *B. bonariensis*. During the Miocene era, successive marine transgressions of the

Atlantic and Pacific Oceans over South America made a maritime channel that separated terrestrial environments. This channel extended over the greater part of Argentina, western Uruguay, southern Paraguay, and southeastern Bolivia (Donato et al. 2003; Donato 2006). This could have resulted in a vicariant process of populations of *B. bonariensis*, possibly isolating terrestrial environments that these organisms inhabited. Additionally, current biogeographical barriers such as the Uruguay River between Argentina and Uruguay, and the Negro River within Uruguay (Fig. 1), could be important causes of divergence among the groups analyzed (Postiglioni & Costa 2006).

Some complete matings between mixed couples from the Buenos Aires and Mendiolaza populations and between the Piedras de Afilar and Mendiolaza populations have been observed (Peretti 1993; Olivero unpub. data). However, some problems during the last stages of mating have been detected (Olivero unpub. data). These problems could be due to some of the morphometric differences in the genitalia between specimens already discussed. Together with the current barriers, this incipient "mechanical incompatibility" could result in a reduction of gene flow between the most distant populations, and each of them could be gathering unique characteristics and becoming differentiated entities. This could be generating speciation, and each group could evolve in an independent way resulting in the differentiation of a new species or subspecies. Nevertheless, it is not possible to confirm this yet, as the genetic structure in each of the species studied is unknown. This generates new questions and shows the need for further studies and genetic analysis, with additional observations of mating behavior between populations, to enrich the present knowledge from phylogeographical and evolutionary perspectives.

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APPENDIX 1

Material of *Bothriurus Bonariensis* Examined.

ARGENTINA: *Córdoba*, Departamento Colón, Mendiolaza, 1995, A.A. Peretti & S. Castelvetri, 1♂ (AVP); 27 January 1996, A.A. Peretti & S. Castelvetri, 1♂ (AVP); 27 December 1996, A.A. Peretti & S. Castelvetri, 7♂, 2♀ (AVP); 8 January 1997, A.A. Peretti & S. Castelvetri, 2♂, 5♀ (AVP); 9 January 1997, A.A. Peretti & S. Castelvetri, 9♂, 4♀ (AVP). *Buenos Aires*, Tandil, 5 February 1945, M.L. Cambio, 1♂ (MACN); February 1947, Prosen, 2♂, 1♀ (MACN); 27–28 November 1967, E. Maury, 3♂ (MACN); 8 December 1969, M.E. Galiano, J. Poirot, 1♂ (MACN); January 1982, M.J. Ramírez, 2♀ (MACN). La Plata, 8 January 1973, C. Cesar, 1♂ (MACN); 18 February 1974, M. Milano, 1♂ (MACN). Gran Buenos Aires, Partido de Ezeiza, 9 January 1969, Gozzi, 1♀ (MACN); Tristán Suárez, 12 February 1997, D. Folgar, 1♂ (MACN); 3 January 1998, C. Velazquez, 1♀ (MACN); 11 January 1998, M. Aberbuj, 1♂ (MACN). Partido de Almirante Brown, Glew, 1974, J. Carpintero, 1♂ (MACN); Adrogué, 5 February 1998, J.M. Morel, 1♂ (MACN). Partido de Escobar Garín, 10 February 1997, E. Maidana, 1♀ (MACN). Partido de Luján, 5 km de Luján, 25 December 1983, I. Vazquez, 1♂ (MACN). Partido de San Miguel, Bella Vista, January 1995, T.M. Gallardo, 1♂ (MACN). Partido de Moreno, April 1998, Rapetti, 1♂ (MACN); Country San Diego, January 1996, M. Lavista, 1♂ (MACN); Country San Diego, 2 February 1998, M. Lavista, 1♀ (MACN). Partido de Pilar, 25 April 1997, D. Castella, 1♀ (MACN). *Entre Ríos*, Departamento Colón Parque Nacional El Palmar, Arroyo El Palmar, 20 January 1967, E. Maury, 1♂ (MACN); April 1974, A. Toth, 2♀ (MACN); February 1981, P. Goloboff, 1♀ (MACN); November 1982, collector unknown, 2♀ (MACN); October–November 2003, collector unknown, 1♂, 1♀ (MACN); Camino a Arroyo El Palmar, 14 December 2005, A. Ojanguren Affilastro, F. Labarque & C. Mattoni, 1♂, 1♀ (MACN); Camino a Arroyo El Palmar, 14 December 2005, A. Ojanguren Affilastro, F. Labarque & C. Mattoni, 4♂, 2♀ (MACN); Camping, under log, 16 December 2005, C. Mattoni, A. Ojanguren Affilastro & F. Labarque, 1♂ (LBRE). URUGUAY: *Departamento Artigas*, Arroyo de La Invernada, under stone, 18 February 1954, San Martín, 1♀ (MACN); under stone, 19 February 1954, San Martín, 1♂ (MACN). *Departamento Rivera*, Ruta 30, km 233, aprox. 100 km S de Artigas, under stone, 345 m, 13 December 2005, A. Ojanguren Affilastro, F. Labarque & C. Mattoni, 7♂, 1♀ (MACN); Ruta 30, km 233, aprox. 100 km S de Artigas, under stone, 13 December 2005, C. Mattoni, A. Ojanguren Affilastro & F. Labarque, 1♂, 1♀ (LBRE). *Departamento Canelones*, Piedras de Afilar: under stone, s/fecha, C. Toscano-Gadea, 1♂, 3♀ (LBRE); under stone, 12 December 2008, P. Olivero, D. Vrech & C. Toscano-Gadea, 4♂,1 ♀ (LBRE).